PB82-223892

Cadmium-Induced Ethylene Production in Bean Plants

Northrop Services, Inc.
Corvallis, OR

Prepared for

Corvallis Environmental Research Lab., OR

25 Sep 81

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1465

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completi)

1. REPORT NO.	2.	3. PB82-223892
EPA-600/J-81-555	Journal Article	

4. TITLE AND SUBTITLE
Cadmium-induced Ethylene Production in Bean Plants

5. REPORT DATE
June 1982

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Northrup Services, Inc. and USEPA, Research Lab
200 SW 35th Street
Corvallis, Oregon 97333

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
same

13. TYPE OF REPORT AND PERIOD COVERED
journal article

14. SPONSORING AGENCY CODE
EPA/600/02

15. SUPPLEMENTARY NOTES
Published in Z. Pflanzenphysiol. Bd. 105, S. 65-74, 1981

16. ABSTRACT

Studies were conducted to (1) compare stress ethylene production from roots and shoots (2) determine the association between stress ethylene production and tissue Cd levels; and (3) investigate the time course of stress ethylene production following the rhizosphere application of cadmium chloride solutions. The shoots and/or roots of bush bean plants (Phaseolus vulgaris L.cv. Bush Blue Lake 290) were encapsulated at specific time intervals following $CdCl_2$ application and kept in the dark for 2 h prior to determining ethylene production. Cadmium accumulated more in the roots than in the shoots and ethylene production was likewise higher in the roots. Cadmium chloride (10 mM)-induced stress ethylene production increased rapidly, peaked within 5 to 10 h and declined to ~0 levels within 24 h after treatment. A subsequent application of 10 mM $CdCl_2$ at 24 h elicited a similar ethylene response, indicating that the plants retained functional ethylene metabolism. Consecutive daily applications (12 days) of $CdCl_2$ (0.1 or 0.5 mM) included only small increases in ethylene production. In all studies however, tissues accumulated large amounts of Cd. Significant increases in ethylene production after a single $CdCl_2$ application were associated with Cd concentrations of ≥ 6 ug g^{-1} dry shoot tissue. The association between stress ethylene production and tissue Cd levels was lost as stress ethylene production declined and also in the consecutive application. The decline in stress ethylene production was attributed to Cd-sequestering which removed the Cd stress.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to public	Unclassified	
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (9-73)

EPA-600/J-81-555
Journal Article

Überreicht vom Verfasser · Nicht einzeln im Buchhandel!
Sonderdruck aus «Zeitschrift für Pflanzenphysiologie», Band 105, Heft 1, S. 65–74 (1981)
Gustav Fischer Verlag Stuttgart

Cadmium-induced Ethylene Production in Bean Plants

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Received July 29, 1981 · Accepted September 25, 1981

Summary

Studies were conducted to (1) compare stress ethylene production from roots and shoots; (2) determine the association between stress ethylene production and tissue Cd levels; and (3) investigate the time course of stress ethylene production following the rhizosphere application of cadmium chloride solutions. The shoots and/or roots of bush bean plants (*Phaseolus vulgaris* L. cv. Bush Blue Lake 290) were encapsulated at specific time intervals following $CdCl_2$-application and kept in the dark for 2 h prior to determining ethylene production. Cadmium accumulated more in the roots than in the shoots and ethylene production was likewise higher in the roots. Cadmium chloride (10 mM)-induced stress ethylene production increased rapidly, peaked within 5 to 10 h and declined to 0 h levels within 24 h after treatment. A subsequent application of 10 mM $CdCl_2$ at 24 h elicited a similar ethylene response, indicating that the plants retained functional ethylene metabolism. Consecutive, daily applications (12 days) of $CdCl_2$ (0.1 or 0.5 mM) induced only small increases in ethylene production. In all studies, however, tissues accumulated large amounts of Cd. Significant increases in ethylene production after a single $CdCl_2$ application were associated with Cd concentrations of $\geq 6\ \mu g\ g^{-1}$ dry shoot tissue. The association between stress ethylene production and tissue Cd levels was lost as stress ethylene production declined and also in the consecutive application study. The decline in stress ethylene production was attributed to Cd-sequestering which removed the Cd stress.

Key words: Phaseolus vulgaris, cadmium, ethylene, stress ethylene.

Introduction

In plants, stress ethylene production is induced by various air pollutants and inorganic and organic compounds applied to either foliage or roots (Tingey, 1980; Rodecap and Tingey, 1981; Thompson et al., 1981). However, little is known about the sites of stress ethylene production. Ozone enhanced ethylene production from foliage but not roots after exposure. The effect of the ozone stress was evidently not translocated throughout the plant as ozone cannot diffuse any significant distance into the growth medium (Tingey, 1980). However, various root stresses can increase shoot ethylene production (Gentile and Matta, 1975; Jackson and Campbell, 1975; Bradford and Dilley, 1978; Jackson et al., 1978; Bradford and Yang, 1980), although it is not clear if the increase results from translocation of ethylene gas, its precursors, or stressant with subsequent ethylene production in the foliage. In tomato plants subjected to flooding, ethylene was transported from roots to shoots where it was asso-

ciated with petiole epinasty (Jackson and Campbell, 1975). Similarly, shoot ethylene production was increased after the roots of tomato plants were immersed in $CuSO_4$ solutions (Gentile and Matta, 1975) or subjected to low O_2 concentrations or anaerobic conditions (Bradford and Dilley, 1978; Jackson et al., 1978). Subsequent flooding studies showed that l-aminocyclopropane-l-carboxylic acid, the immediate precursor of ethylene, was synthesized in the root and transported to the shoot, where it was converted to ethylene (Bradford and Yang, 1980).

Tissue concentrations of Cd and other metals have been positively associated with their solution concentrations (Carlson et al., 1975) and also with metabolic changes, such as reduced photosynthesis (Carlson et al., 1975) and growth (Jastrow and Koeppe, 1980). However, stress ethylene production induced by cadmium and other metals (Rodecap and Tingey, 1981) has not yet been associated with the tissue concentrations of the metals.

The studies reported here were conducted to (1) compare stress ethylene production from roots and shoots; (2) determine the association between stress ethylene production and tissue Cd levels; and (3) investigate the time course of stress ethylene production following rhizosphere applications of cadmium chloride.

Materials and Methods

Ethylene Production From Roots and Shoots

Beans (*Phaseolus vulgaris* L. cv. Bush Blue Lake 290) were greenhouse-cultured in vermiculite in Super Cells, plastic tubular containers with a volume of 165 cm^3 (Ray Leach «Cone-Tainer»

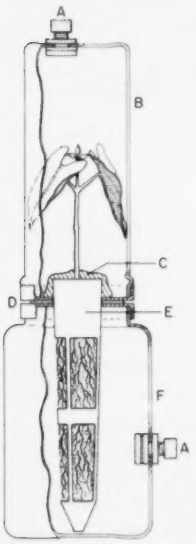

Fig. 1: A cutaway view of encapsulation apparatus for determination of root and shoot ethylene production from intact plants: (A) sampling port; (B) 1-liter plastic container; (C) modeling clay; (D) container lids glued back-to-back; (E) modified Super Cell; (F) 2-liter polyethylene container.

Nursery, Canby, Oregon, USA). A modified Super Cell with 48 % of the surface area removed (125 cm²) was placed inside an unmodified Super Cell. Other growing conditions have been previously described (Rodecap and Tingey, 1981). Plants were exposed to $CdCl_2$ 1 day after the mean plastochron indices for the populations (20 mm control leaf length) were 1.35 ± 0.15 (Maksymowych, 1973; Rodecap and Tingey, 1981). All plants received approximately 4 h of light before solutions were applied. Cadmium chloride solutions and control solutions (reverse osmosis water) were adjusted to pH 6.5 ± 0.1 and applied to the rhizosphere as described by Rodecap and Tingey (1981). After treatment, plants were left in the greenhouse for 5 h. The outer Super Cells were then removed and the roots and shoots of intact plants were encapsulated for 2 h as shown in Fig. 1.

Time Course Experiments

Beans were grown in standard 4-inch (10-cm diameter) pots and exposed to $CdCl_2$ 1 day after the mean plastochron indices for the populations (200 mm control leaf length) were 0.80 ± 0.10. Other cultural conditions, the $CdCl_2$ application procedure, and the encapsulation of individual plants in plastic bags supported by wire frames have been previously described (Rodecap and Tingey, 1981).

Treated and control plants remained in the greenhouse for specified times ranging from 0 to 48 h before encapsulation. A second $CdCl_2$ or H_2O application was administered to some plants 24 h after the initial application. Other plants received applications of $CdCl_2$ or H_2O for 12 consecutive days beginning 12 days after seeding. Each day, four plants from each treatment group were removed for ethylene and tissue sampling 5 h after the $CdCl_2$ had been applied.

Ethylene Accumulation, Sampling and Cd Analysis

To accumulate the ethylene, encapsulated plants were dark-incubated for 2 h at 26 ± 1 °C. Plants were dark-incubated because studies with ozone indicated that dark-incubated plants produced more ethylene than light-incubated plants (Tingey et al., 1976) and also to better control the temperature within the encapsulation containers. Ethylene was sampled from the encapsulation container with a 1-ml gastight syringe, separated on a Porapak N column (2.44 m × 3.2 mm) and quantified with a flame ionization detector (Rodecap and Tingey, 1981).

Tissues were harvested immediately after ethylene sampling, oven-dried at 70 °C to constant weight, ground, digested with 2 % perchloric acid in a block digestor, and analyzed for cadmium by inductively coupled plasma-atomic emission spectrometry (McQuaker et al., 1979). Roots were cleaned by rinsing away the vermiculite with water.

Variances of the ethylene measurements and tissue Cd measurements were positively correlated with the mean ethylene concentrations and tissue Cd concentrations, respectively. The data were transformed to the respective natural logarithms to stabilize variability (Snedecor and Cochran, 1968; Rodecap and Tingey, 1981).

Results

Ethylene Production From Roots and Shoots

Roots produced more ethylene than shoots (Table 1). Cadmium stimulated ethylene production in both roots (10×) and shoots (5×). The pattern of cadmium accumulation in roots and shoots of stressed plants was similar to that of stress ethylene production (Table 1). Cadmium chloride-treated plants accumulated 181 times more Cd in their roots than in their shoots.

Table 1: Stress ethylene production from roots and shoots[a]).

$CdCl_2$ Conc. (mM)	Ethylene nl g^{-1} dry weight tissue		Cd µg g^{-1} dry weight tissue	
	Shoots	Roots	Shoots	Roots
0	37.5 [1.46]	151.2 [1.59]	< 1	< 1
10	188.7 [2.52]	1501.1 [1.35]	14.3 [1.54]	2586 [1.25]

[a]) Geometric means and standard geometric deviations, in parentheses, for ethylene and Cd were based on 24 and 12 observations, respectively. Roots and shoots of intact plants were encapsulated separately. The roots and shoots of all plants contributed 34 and 66 %, respectively, of the plant dry weights.

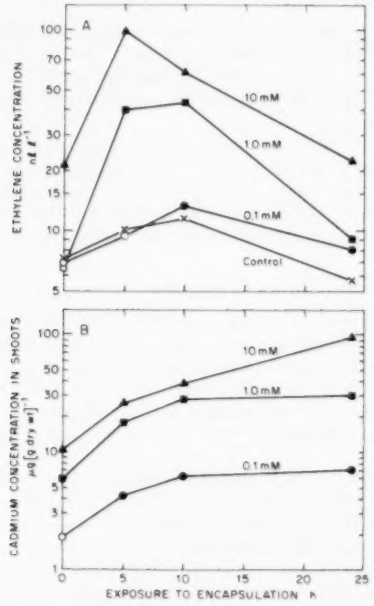

Fig. 2: Ethylene concentration (A) and shoot cadmium concentration (B) as a function of time to encapsulation following rhizosphere application of several $CdCl_2$ solutions. Solid symbols indicate significant increases in ethylene production and Cd tissue concentrations above control levels (t-tests, $\alpha = 0.05$). Each geometric mean was based on 16 observations. The pooled standard geometric deviations for ethylene and Cd were 1.80 and 1.44, respectively. Control Cd levels in the shoots were < 1 µg Cd per g dry weight tissue. Figure 2 A was previously published (Tingey, 1980) but included to allow comparison of ethylene production and cadmium uptake.

Time Course Experiments

Stress ethylene was monitored at specified times to determine the pattern of stress ethylene production. Ethylene production induced by 10 mM $CdCl_2$ was 3-fold

greater than control levels at 0 h. Production increased rapidly after application of 1.0 or 10 mM $CdCl_2$ solutions, reached peak concentrations within 5 to 10 h and thereafter declined to its respective 0 h levels within 24 h (Fig. 2 A). After 0.1 mM $CdCl_2$ was applied, ethylene production was similar to control levels for all sampling periods, although significant increases in ethylene production were measured at 10 and 24 h. In a similar experiment, ethylene was sampled at periods up to 48 h, confirming the response patterns between 0 and 24 h. Ethylene production decreased to ambient levels by 30 h and remained there for the duration of the experiment (data not shown).

The Cd content in the shoots was positively associated with the applied $CdCl_2$ dose and increased with time following its application (Fig. 2 B). However, the Cd uptake rate generally decreased after application except for the uptake rate of 10 mM $CdCl_2$ which was constant between 5 and 24 h.

Stress ethylene production was positively correlated (r = 0.92) with the Cd concentration in the shoots and occurred when the tissue Cd levels were $\geq$ 6 µg Cd per g shoot tissue (Fig. 3). However, the 24-h ethylene-tissue Cd values for the applied Cd concentrations were excluded from the linear regression calculation. The lack of association for the 24 h values was attributed to the decrease in the ethylene production to 0 h levels within 24 h after the $CdCl_2$ application while the shoot Cd concentrations continued to increase.

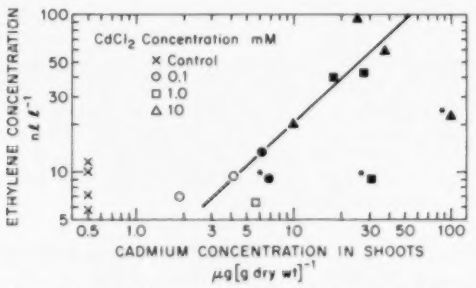

Fig. 3: Ethylene concentration as a function of shoot cadmium concentration. Solid symbols indicate significant increases in ethylene production above control levels. The 24 h data points are denoted by (*). Each geometric mean was based on 16 observations. Plants were the same as in Figure 2.

To determine if the decline in stress ethylene production (Fig. 2 A) resulted from Cd-inactivation of ethylene metabolism, plants were treated with 0 or 10 mM $CdCl_2$ at 0 and 24 h (Fig. 4 A). Ethylene production from the treated plants increased rapidly to a peak value of 88.1 nl l^{-1} at 5 h and then declined to approximately the 0 h value (26.7 nl l^{-1}) within 24 h. A second $CdCl_2$ application at 24 h elicited a similar stress ethylene response. Ethylene production increased to a maximum value of 123.5 nl l^{-1} at 30 h and then decreased to control levels within 48 h after the initial

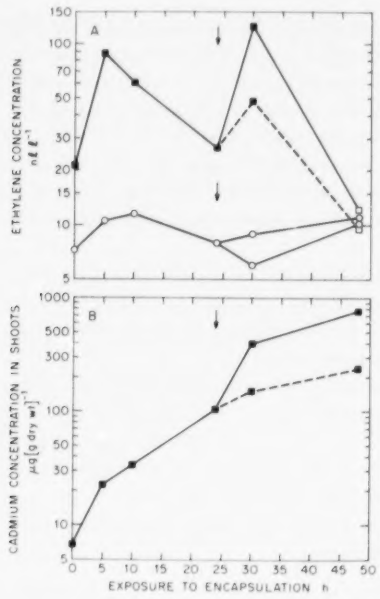

Fig. 4: Ethylene concentration (A) and cadmium concentration of shoots (B) as a function of time to encapsulation following rhizosphere application of $CdCl_2$ solutions. Arrows indicate a second addition of 10 mM $CdCl_2$ or H_2O (- - - - -). Additional controls were run at 30 and 48 h. Solid symbols indicate significant increases in ethylene production and Cd tissue concentrations above control levels (t-tests, $\alpha = 0.05$). Each geometric mean was based on eight observations. The pooled standard geometric deviations for ethylene and Cd were 1.47 and 1.59, respectively. Control Cd levels were < 1 µg Cd per g of tissue.

application. Adding H_2O instead of $CdCl_2$ at 24 h caused a small increase in ethylene production to 48.3 nl l^{-1} at 30 h.

Cadmium uptake was greater following the 24-h $CdCl_2$-application than the 0-h application. This Cd stimulation was consistent with the observations of Petit et al. (1978). Expressed as log-transformed data, however, cadmium uptake patterns are similar after each application. Cadmium levels in the shoots increased at similar rates during the first few hours after each application (Fig. 4 B). The accumulation rates then decreased (5 to 24 h and 30 h to 48 h); however, the rate of decrease was greater between 30 h and 48 h. The ratio of ethylene production to initial cadmium uptake was likewise similar for these periods [0 to 5 h (1.26) and 24 to 30 h (1.20)]. Although water added at 24 h mobilized some Cd that remained in the growth medium from the previous treatment, the Cd increase in the shoots was less than that found after a $CdCl_2$ treatment.

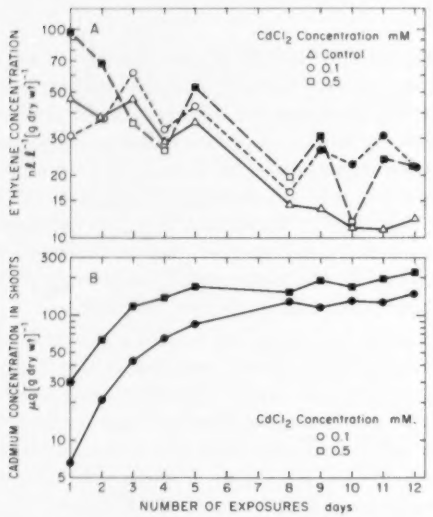

Fig. 5: Ethylene concentration (A) and shoot Cd concentration (B) as a function of the number of exposures to $CdCl_2$. Exposures were conducted once per day. Solid symbols indicate significant increases in ethylene production above control levels (t-tests, $\alpha = 0.05$). Each ethylene geometric mean represents four observations. The pooled standard geometric deviation for ethylene was 1.39. Each Cd value represents a pooled tissue sample from four plants. Control Cd levels were $< 1\ \mu g\ g^{-1}$.

In further studies, plants received a daily rhizosphere application of $CdCl_2$ (0, 0.1 or 0.5 mM) for 12 days to determine if bean plants retained the capacity to produce stress ethylene following repeated stress episodes. Regardless of treatment, younger plants generally produced more ethylene on a dry weight basis than older plants (Fig. 5 A). The measured ethylene concentrations for all treatments were low ($< 25\ nl\ l^{-1}$). These results were expected because the ethylene response induced by 0.5 mM $CdCl_2$ was intermediate to those induced by 0.1 and 1.0 mM $CdCl_2$ in a previous study (Fig. 2 A). However, small but significant increases in ethylene were induced by 0.5 mM $CdCl_2$ after at least six applications, and by 0.1 mM $CdCl_2$ after the final four applications. In contrast to the low ethylene levels, the $CdCl_2$-treated plants accumulated large amounts of Cd (Fig. 5 B). These tissue Cd levels were comparable to those observed in the other time course studies following higher $CdCl_2$ applications (10 mM) and shorter uptake periods (24 or 48 h). The average Cd concentrations in the shoots increased during the first five applications from 6.4 to 85 $\mu g\ g^{-1}$ for the 0.1 mM $CdCl_2$-treated plants and from 29.6 to 170 $\mu g\ g^{-1}$ for the 0.5 mM $CdCl_2$-treated plants. The tissue Cd levels reached an approximate plateau by application eight; following application 12 the levels were 149 and 321 $\mu g\ g^{-1}$ for the 0.1 mM and 0.5 mM $CdCl_2$ treated plants, respectively.

Discussion

The greater accumulation of Cd by bean roots than shoots was consistent with the Cd distribution pattern observed in other plant species (Jastrow and Koeppe, 1980). Cunningham et al. (1975) attributed the cadmium distribution in soybeans to several long-distance transport mechanisms including cation exchange phenomena, diffusion and control by ongoing metabolism. The mobility of Cd within plants also may be related to the solubility of cadmium phosphate (Jarvis et al., 1976; Jarvis and Jones, 1978). Weigel and Jäger (1980) determined that Cd was associated with higher molecular weight components in bean roots than in the shoots, suggesting that the observed distribution may be due to differential translocation of the higher and lower molecular weight organo-Cd complexes within the plants.

Comparing ethylene production per µg tissue Cd for roots and shoots indicated that roots produced only 0.5 nl ethylene compared to 10.6 nl ethylene for the shoots (Table 1). This suggests that roots may be less efficient ethylene producers. The relative inefficiency of root ethylene production may have occurred because the binding of some Cd to root cell walls prevented access to susceptible cellular sites (Ernst, 1980) and subsequent ethylene production. However, after subcellular fractionation of Cd-containing bean tissues, Weigel and Jäger (1980) found that more than 70 % of the Cd was localized in the cytoplasmic fraction of both roots and leaves and only 8 to 14 % associated with the cell wall fractions.

Stress ethylene production was closely associated with tissue Cd accumulation for only 5 to 10 h after $CdCl_2$-application. The association was lost when ethylene production declined while shoot Cd levels continued to increase. Stress ethylene production was related to the Cd content of the shoots because the shoots exhibited a higher efficiency of ethylene production than the roots. The similarities between the stress ethylene responses and also between the Cd-uptake profiles following the two, intermittent $CdCl_2$ applications (Fig. 4) further suggested that increases in ethylene production and the Cd levels of the shoots were positively associated. However, in the daily application study, the Cd-treated plants accumulated large amounts of Cd although only small increases in ethylene production were detected (Fig. 5). This indicated a lack of association between stress ethylene production and shoot Cd content on a long-term basis.

The decline in stress ethylene with time following Cd treatment and the concomitant lack of association between ethylene production and shoot Cd levels may be due to one or more processes such as Cd-inhibition of ethylene metabolism and/or Cd-sequestering thus removing the Cd stress. However, the capacity to retrigger stress ethylene production indicates that Cd did not inhibit ethylene biosynthesis; therefore, another explanation must be found to explain the decrease in ethylene production.

Cadmium-sequestering would allow the cells to recover from Cd-induced perturbations of ethylene metabolism and subsequently permit the retriggering of stress

ethylene production following an additional stress episode(s). Cadmium-binding proteins with properties similar to mammalian metallothionein have been isolated from the roots of Cd-treated tomato plants (Bartolf et al., 1980) and from soybean tissues (Casterline and Barnett, 1977). In addition, most Cd in Cd-treated bean plants was reported to be bound to peptides and/or low molecular weight proteins associated with the cellular cytosol (Weigel and Jäger, 1980). In our studies, Cd-sequestering was suggested by the decline in stress ethylene with time following $CdCl_2$-treatment and the subsequent ability to retrigger stress ethylene production after an additional $CdCl_2$-application. The failure of the plants to produce large amounts of stress ethylene following cumulative low concentration $CdCl_2$ applications while accumulating high levels of Cd in the tissues is also indicative of Cd-sequestering. In this experiment, the plants presumably sequestered Cd before ethylene production could be appreciably perturbed.

Stress ethylene production following rhizosphere applications of $CdCl_2$, foliar O_3 exposures and pathogen inoculation was compared with the tissue accumulation patterns of these stressants. Daily O_3 exposures (4 h) over a 2-week period stimulated stress ethylene production daily (Tingey, 1980); this is similar to the results seen following intermittent applications of $CdCl_2$. However, O_3, in contrast to Cd, does not accumulate in the tissues. On the other hand, stress ethylene production induced by pathogens can continuously increase until an advanced disease state or plant death results. Once inoculated, the pathogen, like Cd, remains in the tissues. *Fusarium*-inoculated pine seedlings, for example, produced ethylene in increasing amounts up to 4 weeks, at which time ethylene production decreased as a result of seedling death (Graham and Linderman, 1980). The similarity of Cd-induced stress ethylene to ozone-induced stress ethylene and not to pathogen-induced stress ethylene further supports the Cd-sequestering hypothesis.

In summary, following a rhizosphere application of $CdCl_2$, roots produced more ethylene and accumulated more Cd than did the shoots. Significant increases in ethylene production were closely associated with the Cd concentration in the shoots. This association was lost as ethylene production declined while tissue Cd levels continued to increase. Decreases in stress ethylene production and the ability to retrigger stress ethylene production after an additional $CdCl_2$-application(s) were attributed to Cd-sequestering in the tissues which removed the Cd stress.

Acknowledgements

The authors thank Mr. Steve Holman, Ms. Marcia Gumpertz and Messrs. Jerry Wagner and Bill Griffis for technical, statistical and analytical assistance, respectively.

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